THE DEVELOPMENT OF THE CYSTOCARP OF GRIF-
FITHSIA BORNETIANA.

ARMA ANNA SMITH.

THE fruiting portion of *Griffithsia* was described in 1861 by Nägeli,[1] who, however, excluded from the genus several of Agardh's species, among them *G. corallina*, which he included under the genus *Heterosphondylium*. The fruiting branch of this genus he described as consisting of a basal joint, bearing a whorl of enveloping branches, a short terminal cell, and one or two intermediate joints, bearing two, or rarely three, trichophores and the favellæ, besides "a characteristic rounded, flattened cell."

Bornet and Thuret[2] in 1867 confirmed Nägeli's observations in regard to the two trichophores borne by the intermediate cell in this species.

Janczewski[3] in 1877 published a detailed account of the development of the cystocarp in the same species, of which a brief summary is here given. The mother cell of the procarp divides by two horizontal walls into three cells, of which the upper undergoes no further development, while the intermediate cell cuts off an anterior cell, which is functionless, and afterwards produces two lateral cells, each of which divides at once into two cells, one small and appendicular (probably Nägeli's "characteristic" cell). The other, which is larger, divides into a "carpogenic cell," which touches the anterior cell, and a mother

[1] Beiträge zur Morphologie und Systematik der Ceramiaceæ; Sitz. d. königl. bayr. Akad. d. Wiss. 2:391–397. 1861.

[2] Recherches sur la fécondation des Floridées; Ann. d. Sci. Nat. Bot. V. 7:147. 1867.

[3] Notes sur le développement du cystocarpe dans les Floridées; Mem. de la Soc. nat. d. Sci. nat de Cherbourg 20:109. 1877.

cell of the trichophoric apparatus, which is turned towards the neighboring vegetative cell.   The trichophoric apparatus consists of four cells with a trichogyne.   After fertilization, all the cells of the procarp gradually die except the carpogenic cell, which increases in size and divides into two cells, of which the upper represents a placenta, giving rise to the lobes of the favella, each consisting of several spores.   Only one trichogyne has been found fertilized in a single procarp.

Dr. Farlow,[4] in The Marine Algæ of New England, published in 1882, says of *G. Bornetiana* Farlow: "In the structure of the procarp this species differs considerably from *G. corallina* as described by Janczewski.   There is only one trichogyne instead of two, as in the last named species.   The procarp begins by the growth of a hemispherical cell at the upper part of an articulation.   The cell is then divided into two parts by a partition parallel to the base.   It is from the lower cell thus formed that the involucre is formed, and from the upper arise the carpogenic cells in the following way: By usually four oblique partitions there are formed four external hemispherical cells and a central pyramidal cell with a broad base.   By subsequent division of one of the hemispherical cells, generally of the one lying nearest the axis of the plant, there is cut off a cell which divides into three smaller granular cells, the upper of which grows into a trichogyne.   The spores are formed by the subsequent growth of the other three hemispherical cells."

As a more detailed account of the development of the cystocarp in this species seemed desirable, the present study was undertaken, the special aim being to learn the method by which the spores arise and the means of transmitting the fertilizing influence from the carpogonium to the cell or cells giving rise to the spores.   In the course of the study several interesting resemblances between this species and *G. corallina* were observed, which have not been published.

The work was carried on under the supervision of Professor Geo. F. Atkinson, to whose assistance in tracing genetic con-

[4] Report U. S. Commission of Fish and Fisheries for 1879.   Washington, 1882.

nections is largely due whatever of value the paper may contain.

The material used in these investigations had been fixed and preserved in a 2 per cent. solution of formalin in sea water. When needed for study, it was stained for an hour in a 1 per cent aqueous solution of acid fuchsin, then washed in fresh water, which was gradually replaced by glycerin, used as the mounting medium. In such preparations it was usually possible to detect the strand of protoplasm passing from a cell to its daughter cell, even though the two were somewhat separated. In some cases, when the connection had been completely broken by accident after mounting, the origin of a cell could be determined by the corresponding portions still projecting from it and from its mother cell. All the important conclusions given in this paper, however, are based upon connections still intact.

The thallus is normally branched dichotomously, and the fruiting branch forms one arm of a dichotomy, arising from the apex of a joint in the upper portion of the shoot. As the corresponding vegetative joint soon exceeds it in size and gives off at its apex, in turn, a fruiting and a vegetative branch, the latter often continuing the process, a series of apparently lateral cysto-carps is thus formed. No uniformity in the relative positions of vegetative and fruiting branches was observed, the cystocarp being in some cases on opposite sides of the corresponding vegetative cell in succeeding joints, sometimes on the same side.

After one fruiting branch has arisen, one usually occurs at each succeeding joint, but two or three sterile joints were sometimes found intervening, without branching. No case was observed in which, after the production of a fruiting branch, further branching resulted in only vegetative joints.

Although the normal branching is dichotomous, cases of trichotomy were observed several times, either three sterile joints arising from the apex of one joint, or a fruiting branch accompanied by two sterile branches.

Judging from the preparations studied, the mother cell of the fruiting branch is cut off before the corresponding vegetative

cell, but the latter appears before the first division in the former, a two-celled fruiting branch being accompanied by a corresponding vegetative one. When, in rare cases, an older fruiting branch was found alone and apparently terminal, as in *fig. 3*, it was not always possible to determine whether it was an abnormal state, or whether the sterile cell had been severed by accident.

The mother cell of the fruiting branch is hemispherical, and is richer in protoplasm than the joint from which it arises. It is soon divided by a wall parallel to its base into a flattened, more or less irregular, hemispherical upper cell and a lower irregularly oblong cell.

A cell is now cut off from the upper cell by a wall oblique to the axis of the branch, as is seen in *fig. 3*. When viewed in some positions this wall appears parallel to the first, as is shown in *fig. 4*. Careful focusing in this case, however, showed that the third cell lies partially under its mother cell and is separated from it by an oblique wall. This may be the terminal cell of Nägeli and Janczewski. It lies in this particular case, however, on the side of the branch towards the vegetative cell, while Janczewski, judging from his figures, though he does not explain his view point, terms cells in this position lateral, and these, according to him, arise after the terminal and the anterior cells in *G. corallina*.

Spalding[5] calls attention to the "irregularity in both the number and position of peripheral cells in *G. Bornetiana*." The present study has confirmed his conclusion, and hence has made the use of such terms as lateral and anterior appear of doubtful propriety in this species, at least.

Fig. 5 represents the same stage as *fig. 4*, but with the vegetative cell at one side. Here the first two peripheral cells are plainly "lateral" according to Janczewski's use of the term, but this was not the case in all the preparations studied.

Before the appearance of a third peripheral cell, one of the two first cut off usually divides into two cells, of which the

[5] Development of the sporocarp of Griffithsia Bornetiana. Abstract; Proc. A. A. A. S. **39**: 327. 1890.

upper is the smaller (*fig. 6*). A third peripheral cell is now cut off, after which a second peripheral cell frequently divides into two cells, making at this time two two-celled branches and one of one cell arising from the central cell. The term central cell in this paper, as in the one quoted,[6] refers to the second cell of the fruiting branch, from which the peripheral cells are cut off, not to the cell giving rise to the spores, to which the term has been applied by Schmitz[7] and Hauptfleisch,[8] but which is here designated as the placental cell, a name used in this sense by Janczewski. This seems to be the normal order, although cases were frequently observed in which only one of the branches consisted of two cells, while in some cases no "appendicular" cell was found in the entire branch. Dr. Farlow does not refer to this division of part of the peripheral cells, and whether it is not a constant feature in this species, as Janczewski holds it to be in *G. corallina*, or whether the appendicular cell, being specially liable to be severed in manipulation, is thus frequently lost sight of, is not easily determined.

The fourth peripheral cell, to which both Farlow and Janczewski refer, was in no case observed in the earlier stages, and its presence was not demonstrated in later ones. This coincides with Spalding's conclusion in regard to the variability in the number of these cells.

The peripheral cells usually present a flattened hemispherical form in optical section, with beak-like projections of protoplasm where they join the mother cell. When lying over the mother cell, the outline is broadly elliptical. They remain smaller than the central cell, which can readily be distinguished until nearly the last stages of the developmental process by its size and its tetrahedral form, produced by the oblique walls

[6] Farlow, l. c.

[7] Untersuchungen über die Befruchtung der Florideen; Sitzb. d. könig. pr. Akad. d. Wiss., Berlin,—: 215. 1883. English translation of same by W. S. Dallas; Ann. and Mag. Nat. Hist. **13** :1. 1884.

[8] Die Fruchtentwickelung der Gattungen Chylocladia, Champia, und Lomentaria; *Flora* **75** : 307. 1892.

meeting at the apex. The change in its contents by which it is also made conspicuous in the later stages will be described later.

One of the peripheral cells first produced, which has already cut off an upper sterile cell, now grows rich in protoplasm and becomes the supporting cell of the carpogenic branch, cutting off the basal cell at one side, but apparently not always on the same side, as may be seen by comparing *figs. 8* and *9*. In *fig. 8* two of the peripheral cells have cut off each a sterile cell. The third peripheral cell has been severed from its mother cell, but the point of former contact may still be distinguished on each cell. In *fig. 9* the mother cell of the carpogenic branch has divided into two cells, which are still in very close connection. Here the supporting cell of the carpogenic branch is the only one with an "appendicular" cell, and as neither of the others shows indications that one has been broken off, it is probable that no other has been produced.

In none of the mature carpogenic branches observed were fewer than four cells found. The branch is curved so as to form almost a right angle at the second cell (*figs. 10–13*). The terminal cell, the carpogonium, is smaller than the others, nearly triangular in outline, and prolonged into a trichogyne from the pointed apex.

The trichogyne is an elongated slender structure, as long as the remainder of the fruiting branch, and is straight or only slightly curved, lying in a direction parallel with the axis of the branch. Its diameter is slightly greater at the apex than in the portion adjoining the carpogonium. Its contents do not take the fuchsin stain, but a decidedly granular appearance is observable.

In *fig. 11* a peculiar development is shown, and a somewhat similar, though not quite identical, appearance was observed in another case. The supporting cell of the carpogenic branch has given rise, not to a single sterile cell, but to a branched cellular filament. The carpogonium is slightly larger than usual, and no trace of a trichogyne is seen. It seems probable that in

default of fertilization the disappearance of the trichogyne has been followed by vegetative growth in the carpogenic branch and by the production of extra vegetative cells elsewhere. Here, too, there seem to be four peripheral cells, though the relations of the two cells lying below the central cell were not positively determined.

The process of fertilization and its immediate effects were not observed, lack of suitable material making a nuclear study impossible. In *fig. 12* a pollinoid is shown in contact with a trichogyne, which is enlarged at the point of attachment, but how far fusion takes place was not ascertained.

After fertilization, the trichogyne gradually withers and soon disappears, the basal portion remaining for a time attached to the carpogonium. The latter loses its contents, assumes an irregular form, and becomes disorganized. The adjacent cell at the same time apparently increases in size, but it also soon loses its contents, and in some cases appears to become disorganized, while the two lower cells take a deeper stain than before. Whether the intervening walls are absorbed or a transfer of protoplasm takes place through the enlarged pit connections was not determined, but it is evident that with the disorganization of the upper portion of the carpogenic branch, the supporting cell and the central cell grow rich in contents, the latter especially at this time taking a very deep stain with fuchsin. The carpogonium does not persist long after fertilization, either individually or as a part of a large fusion cell.

Schmitz (l. c.) includes *Griffithsia* among the genera in which conjugation probably takes place between the carpogonium and the supporting cell, which thus becomes the auxiliary cell. He does not state, however, that he has observed the process in any species of the genus. The two cells do not always lie sufficiently near together at the time of fertilization to allow direct conjugation, and no evidence was seen either of change of position or of the growth of ooblastema threads.

In no case was any indication of spore production seen until after a gradual loss of contents in the upper cells of the car-

pogenic branch, which seemed to be followed by changes in the appearance in the lower ones, both following upon fertilization.

At this stage the supporting cell, now rich in protoplasm, increases in size and cuts off a cell, which, in turn, becomes larger, though without taking a deeper stain, and becomes the placental cell. From all sides of this cell, cells arise which are distinguishable at once by their large nuclei, and it is by their subsequent division that the lobes of spores arise, each cell thus cut off from the placental cell developing into one lobe. Each cell cuts off small cells from the sides and end, and soon assumes a cuneate form, the individual segments having a similar or a clavate form. Each lobe consists for a time of a compact mass of cells, but as the cells separate and become rounded off as spores, it develops into a branched tuft made up of loosely connected chains of ovoid or elliptical spores, developed in basipetal succession, each with a large nucleus. The process, however, is apparently not strictly uniform in all cases, as may be seen by comparing *figs. 15–19.* The lobes arise successively, and thus different stages of spore formation may be seen in a single preparation (*figs. 15–19*). According to Harvey[9] each lobe constitutes a favella, and thus several favellæ are contained within a single involucre, while Farlow regards the lobe collectively as constituting one favella.

The favellæ, as well as the vegetative cells of the thallus, are surrounded by gelatinous membrane, which has been omitted in the figures for the sake of clearness. Around the vegetative cells it is so swollen by the glycerin as to be very evident, but it often remains in such close contact with the lobes of spores as to be detected with difficulty.

Directly after fertilization, as the central cell begins to take a deep stain, the growth of the involucral branches begins. The basal cell of the fruiting branch cuts off cells, which remain small and oblong, but from each of which another cell is cut off,

[9] Nereis Boreali-Americana **2**: 227. 1853. Smithsonian Contributions to Knowledge No. 5.

which grows rapidly, assumes at first an obovate, later an elliptical form, slightly reniform, and a length greater than that of the cystocarp. The cells form thin, translucent, expanded plates, and although they completely envelop the favellæ, the cells within may be studied, the details, however, being greatly obscured by them. Seven of these involucral branches were counted in one case, but the number varies. Nägeli and Janczewski call attention to the bicellular character of these branches in *G. corallina*, which, the former states, had been overlooked by systematists, and to which the present writer has found no reference in published descriptions of *G. Bornetiana*.

Although the later stages in the development of the cysto-carp are made out with difficulty, the placental cell can be seen to stretch out and become irregular in form, and it seems highly probable, though not quite certain, that fusion takes place between it, the supporting cell, and the central cell, forming one large placenta, the lobes arising from the portion corresponding to the original placental cell. *Fig. 19* is drawn to the same scale as the others, and thus shows not only the great irregularity in the form of this fusion cell, but also the great increase in size over any cell previously met with in the cystocarp. It is diffi-cult to determine the exact nature of the large cells joined to it, but they seem to be sterile cells unconnected with the sporogenous cells, which for some reason have taken on this increase in size.

No evidence was found that the peripheral cells other than the supporting cell of the carpogenic branch have a part in spore production. They gradually become inconspicuous after fertili-zation, and finally disappear with the carpogenic branch and the sterile cell given off from the supporting cell, unless the branch referred to in the preceding paragraph and represented in *fig. 19* is made up of such cells which have taken on vegetative growth. It is easier to conceive the influence of fertilization as transmitted to the supporting cell through the cells of the car-pogenic branch than to the other two or three peripheral cells,

either through the central cell or by separate conjugation with the carpogonium.

The development of the cystocarp in this species thus resembles very closely that of *G. corallina* as described by Janczewski, with the single exception of the one carpogenic branch instead of two. He does not speak, either, of the fusion forming a large central cell, though one of his figures (7) suggests that it may take place.

The process has its more or less perfect analogy in related genera also. In *Ceramium decurrens*, according to Janczewski, the supporting cell, which, after cutting off a sterile cell, gives rise to two trichophores, becomes the "carpogenic cell," cutting off an upper placental cell after fertilization. Schmitz concludes that here conjugation probably takes place between the carpogonium and the supporting cell, but does not claim to have observed it. Janczewski represents the two cells as lying adjacent, and so this fusion would be more easily accomplished than in *Griffithsia Bornetiana*, where they are not always close together, though they are in some cases, as is seen in *fig. 10*.

Phillips[10] states of *Rhodomela subfusca*, *Polysiphonia nigrescens P. fastigiata*, and *P. violacea* that the second cell of a branch cuts off five pericentral cells, one of which, besides serving as supporting cell of the carpogenic branch, gives off two sterile branches, one of two cells and one of one cell, and then becomes the auxiliary cell, cutting off, after fertilization, an upper sporogenous cell. In these cases the carpogenic branch is so curved that the carpogonium lies close to the auxiliary cell, and both Schmitz and Phillips conclude that conjugation takes place between the two, though neither of them actually observed the process. According to Phillips, fusion takes place between the sporogenous cell, the auxiliary cell, and its sterile branches in *P. nigrescens* and *P. fastigiata*, while in *P. violacea*, as apparently in *Griffithsia Bornetiana*, the central cell is included in the fusion.

[10] On the development of the cystocarp in the Rhodomelaceæ, Ann. Bot. **9**: 289. 1895.

In connection with this study the question naturally arises as to the proper limitations in the use of the term "auxiliary cell." Schmitz applies it to cells performing slightly different functions. 1. To a cell of the thallus or of the carpogenic branch which grows rich in protoplasm and then gives up its contents to an ooblastema thread, thus supplying it with the extra nutriment necessary for the production of spores, as in *Naccaria* and *Petrocelis*. 2. To a cell which enters into conjugation with the fertilized carpogonium or with a filament arising from it, and which after thus receiving the influence of fertilization, itself cuts off a cell from which the sporogenous filaments arise, as observed by him in *Glæosiphonia*. It is in this sense that he applies the term to the supporting cell in *Griffithsia*, assuming that conjugation takes place between it and the carpogonium, either directly or by means of a short conjugation process. Mr. Davis,[11] in his description of *Champia parvula*, applies the term to ordinary thallus cells, which, so far as he has observed, do not fuse with any of the cells directly concerned with the development of the cystocarp, but which give rise to the wall of the cystocarp, after a modification of their contents and union with one another and with the supporting cell by means of protoplasmic processes. It seems very doubtful if such a use of the term is in accordance with Schmitz's application of it. It would seem much less questionable to apply it in *Griffithsia* to the supporting cell, which gives rise to the placental cell, even though the influence of fertilization is transmitted through the cells of the carpogenic branch by a broadening of pit connections or the absorption of walls instead of by means of conjugation between the carpogonium and the supporting cell. Janczewski following Bornet and Thuret applies the term carpogenic cell to the cell which gives rise to the spores, whether it be the carpogonium, which these writers regard as a part of the "trichophoric apparatus," or some other cell which performs the function of spore production. Later writers limit the application of carpogenic cell to the cell directly beneath the trichogyne, whatever its fate after fertiliza-

[11] Development of the cystocarp of Champia parvula, BOT. GAZ. **21**: 109. 1896.

tion, and there seems to be at present no general term agreed upon for the spore producing cell. The study of the exact process of fertilization and its effects in a greater number of species is necessary before the question of homologies, and hence of terminology, can be definitely settled.

Besides the greater complexity and the presence of the enveloping cells, which render it more difficult to trace the later stages of the development of the cystocarp in *Griffithsia Bornetiana*, the obscurity is increased by the presence in large numbers of dichotomously branched hairs, which sometimes nearly cover the cystocarp. Dr. Farlow calls attention to these hairs arising from the upper border of the thallus cells. In the present study they were never found elsewhere than at the upper portion of a joint, and, with one exception, never on other joints than those bearing cystocarps. In that case it seemed possible that a cystocarp had been broken off, though that could not be demonstrated. Although a cystocarp sometimes occurs without these hairs their presence was found to be so nearly constant as to suggest a possible connection between the two, either morphological or physiological.

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EXPLANATION OF PLATES I AND II.

All the figures were drawn with the aid of the camera lucida from material stained with acid fuchsin and mounted in glycerine.

j. Joint from which the fertile branch has arisen. *b.* Basal cell of fruiting branch. *c. c.* Central cell. *p.* Peripheral cell. *st.* Sterile cell cut off from peripheral cell. *s.* Peripheral cell functioning as supporting cell of carpogenic branch. *c.* Carpogonium. *t.* Trichogyne. *pl.* Placental cell. *l.* Sporogenous lobe. *sp.* Spore. *b. i.* Basal cell of involucre filament. *i.* Terminal cell of involucre filament. *v.* Neighboring vegetative cell.

FIG. 1. One-celled fruiting branch, somewhat separated from main joint, but connected by a stout strand of protoplasm.

FIG. 2. A two-celled branch.

FIG. 3. The first peripheral cell has been cut off from the central cell by an oblique wall. The vegetative cell has not developed or has been severed.

FIG. 4. The four-celled branch lies over the vegetative cell. One peripheral cell lies partially beneath, the other above the central cell.
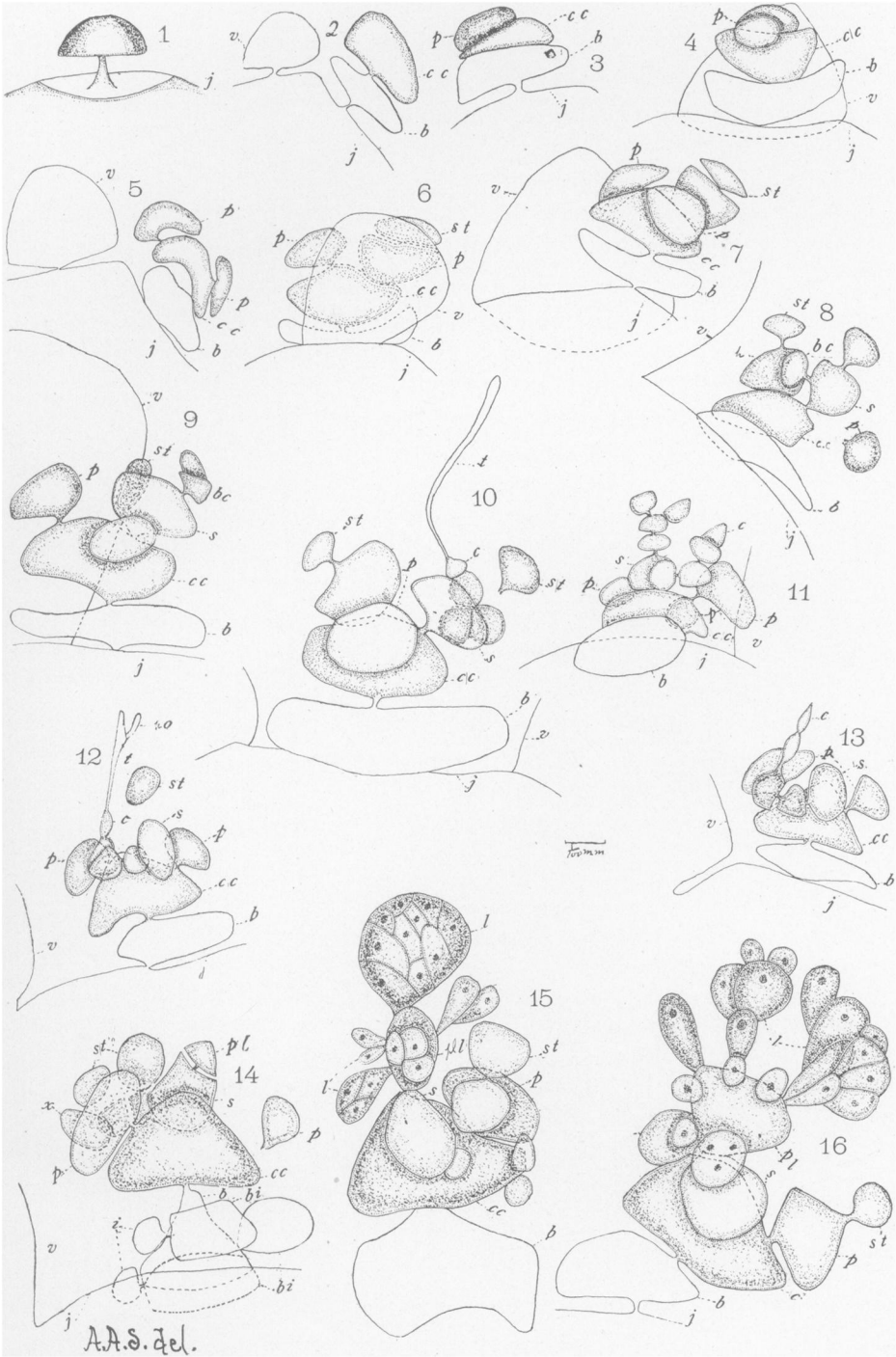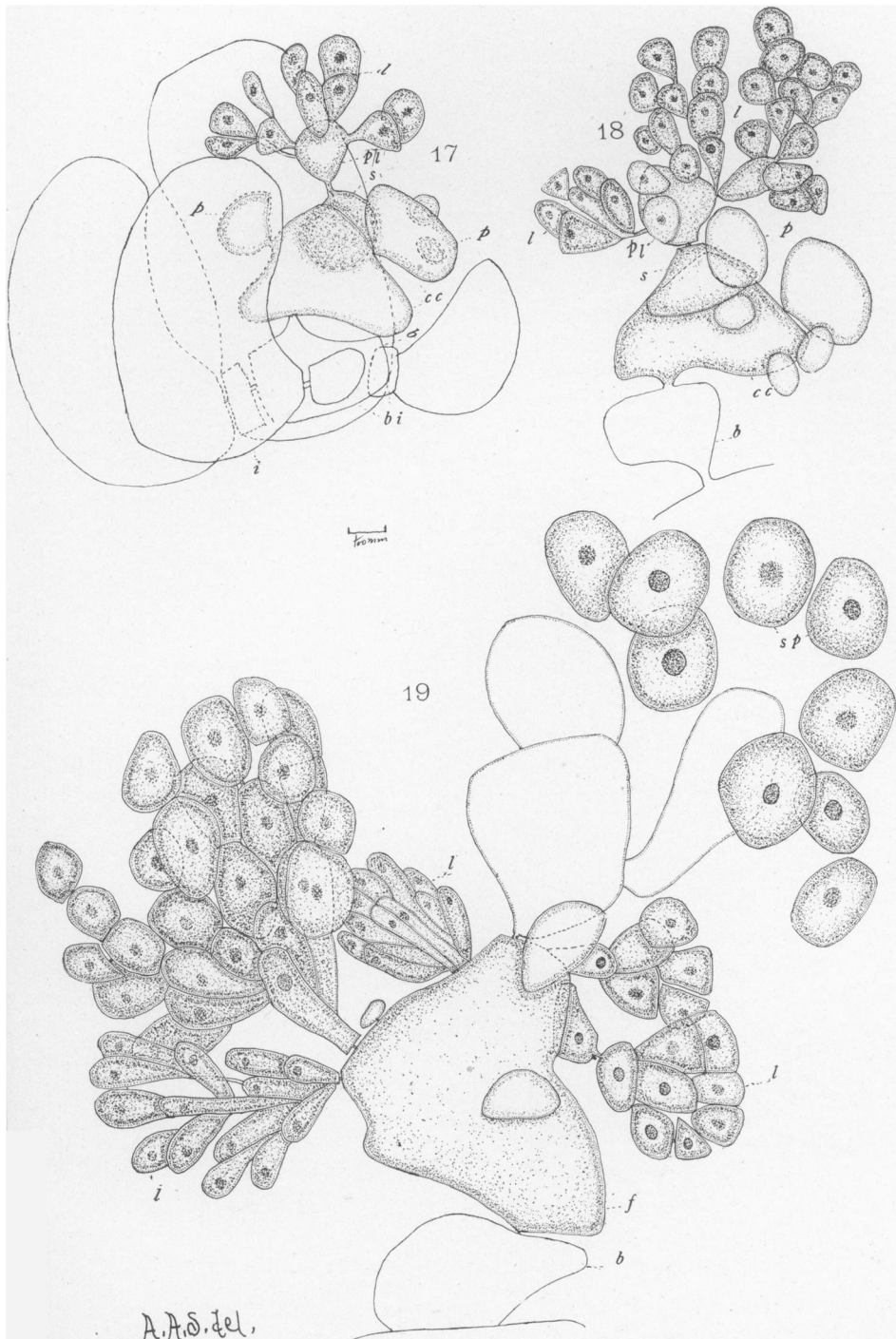
SMITH on GRIFFITHSIA.

FIG. 5. A different view of a stage similar to that represented in *fig. 4.*

FIG. 6. The branch lies under the vegetative cell. One of the peripheral cells has cut off a sterile cell.

FIG. 7. The third peripheral cell has been cut off from the central cell.

FIG. 8. Two peripheral cells have divided, and the supporting cell has produced the basal cell of the carpogenic branch (*b. c.*). One peripheral cell has been severed from its mother cell.

FIG. 9. The carpogenic branch now consists of two cells. The second peripheral cell has no appendicular cell.

FIG. 10. The entire fruiting branch lies above the corresponding vegetative cell. The carpogenic branch has reached maturity, and the carpogonium is prolonged into the trichogyne.

FIG. 11. An abnormal vegetative development from the supporting cell.

FIG. 12. A pollinoid (*p.*) is attached to the trichogyne. Penultimate cell of the carpogenic branch large and lying partially beneath the second cell.

FIG. 13. The trichogyne has disappeared, and the carpogonium and penultimate cell have lost their contents and shrunken. The second cell of the carpogenic branch, as well as the basal cell, is rich in protoplasm.

FIG. 14. The supporting cell and the central cell have abundant protoplasm. The former has cut off the placental cell. The meaning of x is uncertain, its connection with any other cell not being clearly made out. The involucral filaments have begun to develop.

FIG. 15. The entire branch has increased in size. The carpogenic branch has become disorganized, the basal cell remaining attached to the supporting cell. Several lobes are arising from the placental cell, and are seen in different stages. Involucral threads omitted to avoid confusion.

FIG. 16. A similar or slightly later stage than *fig.* 15. Involucral threads again omitted.

FIG. 17. The young cystocarp inclosed in the involucre. Three of the filaments are fully grown ; one is still small.

FIG. 18. A slightly later stage with involucre omitted.

FIG. 19. A still later stage. Fusion has apparently taken place between the placental cell, supporting cell, and central cell, and lobes are arising from the large fusion cell (*f.*). Some of the spores are mature, and their connections have been broken. Most of the sterile cells have disappeared, while some seem to have developed abnormally.